THE PERFUSION EXPERIMENT IN THE STUDY OF CELLULAR ANAPHYLAXIS *

WITH ONE PLATE

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Although a large amount of work has been done in recent years in attempts to determine the fundamental nature of anaphylaxis, this problem has not been entirely cleared up. It is not the purpose of this paper to attempt in any way to review the vast literature which has accumulated on the subject. In the interest of context, however, it may be recalled that there are two theories regarding the fundamental mechanism of anaphylaxis. Friedberger and his pupils hold to the humoral theory. According to this conception, when an antigen is acted on by its specific antibody of the third order the antigen is broken up into toxic substances which are called anaphylatoxin or apotoxin, to which all manifestations of protein anaphylaxis are ascribed. This conception of the mechanism of anaphylaxis would seem to be supported by the test tube experiment, in which apotoxin may be readily formed by bringing together the suitable reagents. The humoral theory is, however, wholly inadequate to account for the observation that, if an animal is immunized passively, a certain incubation period must elapse before anaphylactic phenomena manifest themselves on the introduction of the antigen.

It was a study of the mechanism of passive anaphylaxis that led to the development of the cellular theory, the chief proponents of which are Schultz,[1] Dale,[2] Weil,[3] and Cocoa.[4] According to the cellular theory the site of reaction in anaphylactic phenomena is not in the tissue fluids but within the cells. When an animal is immunized passively it is assumed that the tissue cells appropriate the antibodies and thus become sensitized to the antigen. According to the view of the proponents of the cellular theory of anaphylaxis, we have here

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1 Jour. Pharm. and Exper. Ther., 1910, 1, p. 549; Hygienic Lab., Bull. No. 80, 1912.

2 Jour. Pharm. and Exper. Ther., 1913, 4, p. 167.

3 Jour. Med. Research, 1913, 22, p. 497; 1914, 25, p. 87 and p. 299.

4 Ztschr. f. Immunitätsforsch. u. exper. Therap., O., 1914, 20, p. 622.

a plausible explanation of the period of latency required to effect passive sensitization, which the Friedberger theory does not supply. If we accept this explanation of anaphylaxis it is apparent that the tissue cells discriminate between the different orders of antibodies, as passive immunization does not always sensitize against the specific antigen. Were this the case it would be the height of folly to administer tetanus antitoxin prophylactically, as such treatment would sensitize the individual to tetanus rather than afford protection. It is, on the contrary, admittedly good therapeutics to immunize passively against tetanus and in some cases against diphtheria as well. The individual immunized against tetanus never becomes more sensitive, but invariably more immune to tetanus toxin. If the tissue cells should appropriate the injected tetanus antitoxin and the individual later become infected with tetanus, the passive immunization would probably prove fatal. It would therefore appear that antibodies of the first order are not involved in the mechanism of cellular anaphylaxis. The question as to the reason for this discrimination by the tissue cells between the antibodies of the first and third order would indeed be an interesting problem for investigation.

While there are some very potent objections to the unreserved acceptance of the cellular theory of anaphylaxis, it has, on the other hand, much in its favor. The fact that a period of incubation of at least from 4-6 hours is required to sensitize passively would seem to allow of no other explanation. This is further supported by the observation of Weil that tissue sensitization is coincident with the disappearance of antibodies from the blood stream. Weil found further that a sensitized animal could be protected from anaphylaxis by insuring the presence of antibodies in the blood stream when the antigen was given.

The final supporting pillar to the theory of cellular anaphylaxis was furnished by the work of Schultz and Dale who attempted to wash the sensitized tissues free of blood and lymph, after which such tissues were exposed to the stimulus of their specific antigens. These experiments presuppose the ability completely to isolate the tissue cells from the body fluids. Dale washed the uterus of the guinea-pig supposedly free of blood by inserting a cannula into the abdominal aorta and perfusing the lower half of the body with Locke's fluid, allowing the efferent fluid to escape from the inferior vena cava. The perfusion was continued "for upwards of half an hour" until the efferent fluid appeared free of blood. The same technic has been used by Weil and others in the study of cellular anaphylaxis in guinea-pigs.

No attempt was made by these authors to estimate the amount of residual blood remaining in the tissues following perfusion. Such investigation was undertaken by Cocoa. By estimating the amount of blood cells and complement in the perfusion fluid he arrived at the conclusion that from 1.6-6% of residual blood remained in the tissues following perfusion.

Manwaring[5] perfused the lungs of normal and anaphylactic guinea-pigs by putting the afferent cannula into the pulmonary artery and allowing the fluid to escape through an incision in the left auricle. Assuming that he had washed the lungs free of blood with Locke's solution, he concluded that the anaphylactic reaction was partly cellular and partly humoral.

In studying spontaneous phagocytosis in the liver with India ink we were surprised to find that the ink particles were deposited only in restricted areas of the organ. It was evident that only a small part of the liver was traversed by the perfusing fluid. This observation suggested the possibility of making use of this procedure in studying the course of the perfusing fluid through an organ and in determining to what extent it is possible to wash an organ free of blood.

EXPERIMENTS

The apparatus is so constructed that fluid, at body temperature, may be passed through the organ and collected for examination. The animal is placed inside an incubator kept at a temperature of 37 C. A funnel provided with a stopcock is supported on a ring-stand. Fluid from the funnel passes through a glass coil which rests in a beaker of water kept at a temperature of 38 C. A rubber tube leads from the glass coil through a small hole in the side of the incubator to the afferent cannula. This tube is connected with a mercury manometer and the height of the funnel is so adjusted that the manometer registers a pressure of about 2-3 cm. Hg. The efferent cannula is connected with a rubber tube which passes through a small hole in the side of the incubator so that the fluid may be collected outside. Several places are provided to let out any air bubbles which may get into the apparatus. By means of the stopcock on the funnel the perfusion may be discontinued temporarily at any time. Locke's solution, at a temperature of about 37 C., was used to perfuse the organs. Most of the experiments were performed on the liver, but the lungs and the uterus were also perfused a few times each. A typical experiment will illustrate the procedure and the results.

The rabbit is killed with ether. Before the heart quits beating a cannula containing sodium citrate is inserted into the portal vein near its entrance into the transverse fissure of the liver. The gastric vessels in the lesser omentum are then tied off. Another cannula is inserted into the superior vena cava in the thorax. From 50-100 c c of Locke's solution warmed to 37 C. are passed through immediately under gentle pressure to wash out most of the blood. The animal is then put into an incubator (temperature 37-40 C.),

[5] Jour. Immunol., 1917, 2, p. 157.

and the perfusion is begun at once, about 10 minutes after the insertion of the first cannula. The manometer registers a pressure of about 2-3 cm. Hg. Perfusion begins at 2:55. Fluid from exit cannula is reddish at first but finally becomes clear. At 3:07 a faint trace of albumin in fluid from exit cannula; 3:15, albumin negative. Perfusion stopped for 3 minutes, then continued. Fluid is now blood stained, shows erythrocytes microscopically, and gives positive albumin test. 3:22, albumin negative; perfusion stopped for 5 minutes. 3:27, perfusion begun again. Fluid contains blood. Albumin strongly positive. 3:35, albumin negative. Perfusion discontinued for 6 minutes. 3:41, perfusion continued. Blood and albumin present. 3:48, albumin negative. Perfusion discontinued for 5 minutes. At 3:53, perfusion continued. Blood and albumin present. 4:00, albumin negative. Perfusion discontinued for 5 minutes. 4:05, perfusion continued. Blood and albumin present. 4:06, end of experiment. About 2,000 c c of Locke's solution has been passed through the liver.

All perfusions, whether of liver, lung, or uterus, give about the same results. It is never possible to wash out all the blood and albumin. The fluid from the exit cannula soon becomes free of albumin and blood, but if the perfusion is discontinued for a few minutes both these substances reappear. The explanation of this phenomenon will be more apparent after we have considered the experiments with India ink.

TYPICAL INDIA INK EXPERIMENT

A liver is prepared as described above and washed with 1,000 c c of Locke's solution. The fluid from the exit cannula is free from albumin. Then a litle India ink (Higgins) is added to another 1,000 c c of Locke's solution and this is passed through the liver. The ink suspension is very thin. At first the fluid from the exit cannula is clear, all the ink particles having been taken up by the endothelial cells of the liver. Later on the fluid from the exit cannula is just as dark as that entering the afferent cannula, showing that no more ink particles are being held in the liver. The perfusion is discontinued at this point. The pressure is constant throughout the experiment and the rate of flow from the exit cannula is unchanged, indicating that there is no obstruction to the flow of the fluid.

A liver perfused with Locke's solution containing India ink shows blackened portions, the black color being due to retention of the carbon particles of the ink by the endothelial cells of the liver. The blackened areas evidently correspond to the parts of the liver through which the perfusing fluid passed. There is great variation in the size and position of the blackened areas in different specimens. In every case there are large areas of liver tissue not blackened at all. In some instances only the dorsal part of the liver is blackened (this is the lower part during the perfusion); in others only a comparatively small area around the hilus. The blackened areas shown in Figs. 1, 2, 3, and 4 indicate the parts of the liver through which the perfusing fluid actually passed. It is clear that even in the most favorable

cases there are extensive areas of liver tissue not reached by the perfusing fluid and that sometimes only an insignificantly small part of the liver is perfused. Even in blackened portions of the liver the cut surface shows that the fluid has reached only the central parts of the lobule (Fig. 5). It is therefore an error to assume that all the blood has been washed out of the liver because the fluid coming from the organ is free from albumin.

It was pointed out in the foregoing that it is not possible to wash all the blood out of the liver. It is easy to get the fluid from the liver free from albumin by continuous rather rapid perfusion; but if the perfusion is discontinued for a few minutes blood and albumin invariably reappear. The experiments with ink give us a clear explanation of this phenomenon. The perfusing fluid traverses only certain parts of the liver and these soon become free of blood; but during the periods when the perfusion is discontinued, blood from the adjacent areas of liver tissue diffuses into the main path of the fluid. Hence, at each renewal of the perfusion after a pause blood and albumin reappear.

On theoretical grounds it is not to be expected that all of the blood can be washed out of an organ with Locke's solution since the latter is much thinner than blood and produces much less friction on the walls of the vessels. As soon as a passage is opened up through the organ the resistance along this route will be much less than through capillaries containing blood, and the fluid will take the path of least resistance.

Air bubbles cannot be a factor in directing the course of the perfusing fluid since these were always carefully excluded. The ink does not block any of the blood vessels. The carbon particles are found on microscopic examination for the most part in the endothelial cells. A liver blackened by an ink perfusion is unaffected by washing with clear Locke's solution. No carbon particles are washed out.

The lungs were perfused by putting the afferent cannula in the pulmonary artery and the efferent in the left auricle. The trachea was ligatured before the thorax was opened to prevent collapse of the lungs. It was also found impossible to wash all the blood out of the lungs. The efferent fluid soon became free of blood and albumin, but both these substances reappeared after temporary interruption of the perfusion. Perfusion with India ink, after preliminary washing with Locke's solution, showed the same irregular blackening of the lung tissue as was described above in the liver (Fig. 6).

The uterus was perfused according to Dale's method. In general, the same results were obtained as with the lungs and the liver.

Since the perfusion of an organ is so incomplete it is obvious that circulating antibodies are not entirely removed by this procedure. It follows that the experiments of Dale and Weil with perfused uterine muscle cannot be regarded as lending any support to the theory of cellular anaphylaxis. In like manner it will be seen that the presence of cellular antibody is not established in Manwaring's experiment with 14-day anaphylactic lung.

It is recognized by us that our experiments do not overthrow the doctrine of cellular anaphylaxis. It is our purpose only to call attention to the unsoundness of one of the main arguments which have been advanced in its support.

SUMMARY

It has been shown that it is impossible to wash all the blood out of an organ by perfusion methods. The efferent fluid becomes free from blood and albumin in a short time, but these substances reappear if the perfusion is temporarily discontinued a few minutes.

By perfusing organs with Locke's solution containing India ink, the course taken by the fluid through the organ has been mapped out. It has been found that a surprisingly small part of the capillary system is really washed out by the perfusing fluid.

This technic does not therefore remove circulating antibodies completely, as has been assumed, and this type of experiment does not establish the presence of cellular antibodies.

EXPLANATION OF PLATE

The organs were first perfused continuously with warm Locke's solution until the efferent fluid was free from albumin, after which they were perfused with warm Locke's solution to which some India ink had been added. The blackened areas show the course of the fluid through the organ. All figures are photographs.

Fig. 1. Ventral surface of liver.

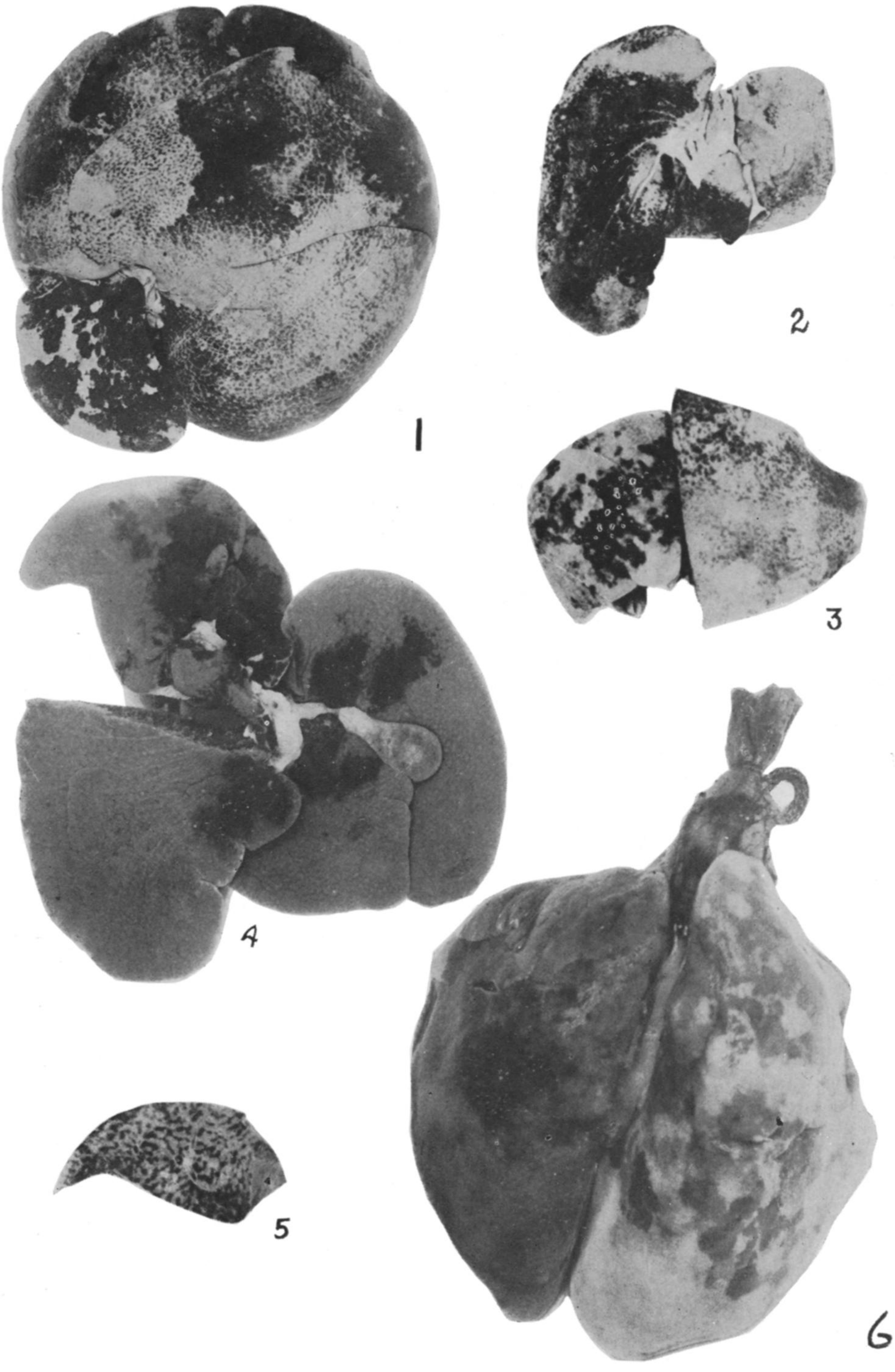
Fig. 2. Dorsal surface of liver. Very little passed through the left lobe.

Fig. 3. Two pieces of a liver, showing a very incomplete perfusion.

Fig. 4. Dorsal surface of liver. The fluid passed through a relatively small area around the transverse fissure.

Fig. 5. Section of a deeply blackened area from a liver, showing that even in this part not over half the hepatic cells are reached by the perfusing fluid.

Fig. 6.—Dorsal surface of lungs. Very incomplete perfusion of one lung.



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